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Suspect screening of natural toxins in surface and drinking water by high performance liquid chromatography and high-resolution mass spectrometry

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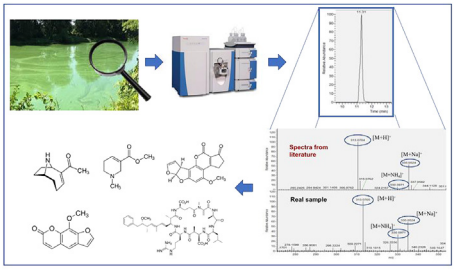
HIGHLIGHTS

- Single workflow for a comprehensive assessment of natural toxins in waters is presented.
- The procedure includes a suspect screening of natural toxins and target screening of a selection.
- The approach tentatively identified 23 natural toxins in the Ter River (Spain).

GRAPHICAL ABSTRACT



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ABSTRACT

Besides anthropogenic contamination, freshwater environments can also be affected by the presence of natural toxins. Mycotoxins, plant toxins and cyanotoxins are the most relevant groups that can be found in the aquatic system. However, until now, only cyanotoxins have been more carefully studied. In the present work, single workflow for the assessment of natural toxins in waters, based on suspect screening and target screening of a selected group of toxins is presented. The approach is based on a triple-stage solid-phase extraction (SPE) able to isolate a wide range of natural toxins of different polarities, followed by liquid chromatography coupled to high-resolution mass spectrometry (HPLC-ddHRMS^2) using a Q-Exactive Orbitrap analyser. The acquisition was performed in full-scan (FS) and data-dependant acquisition (ddMS^2) mode, working under positive and negative mode. For the tentative identification, different on-line databases such as ChemSpider and MzCloud and an in-house natural toxins list with 2384 structures, that includes cyanotoxins, plant toxins and mycotoxins, were used. Also, thanks to the MS^2 data, it was possible to achieve a high level of tentative identification confidence, but confirmation was only possible comparing the standards of the suspected compounds. For those, the analytical parameters of the developed method were also validated, and the quantification was possible by external calibration. Validation showed recoveries in the range between 53 and 95%, and method limits of detection (MDL) between 0.02 and 1.22 μg/L. This approach was applied to study natural toxins in 4 sampling sites along the Ter River in Catalonia (NE Spain). In this preliminary study 23 natural toxins were tentatively identified, and 9 of them confirmed (aflatoxin B1, anatoxin-a, nodularin, microcystin-LR, baicalein, kojic acid, cinchonine, B-asarone and atropine). The results of the quantification of these compounds showed concentrations below 1 μg/L in all cases, that is considered safe according to the

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actual legislation. This suspect screening approach allows a more comprehensive assessment of natural toxins in natural waters.

1. Introduction

The occurrence of natural toxins in the surface and drinking water reservoirs is a problem of increasing concern for water producers and environmental scientists. Natural toxins are secondary metabolites produced by a multitude of organisms and especially by fungi (fungal toxins or mycotoxins), plants (phytotoxins), bacteria and algae. Natural toxins are naturally synthesized by living organisms and it has been hypothesised that defence or predation mechanisms can be some of the factors ruling their production (Clucas et al., 2010). The release of natural toxins into the environment, and in particular their occurrence in surface waters, can constitute a hazard for human health due of possible ingestion and/or recreational and bathing activities (Hodgson and Hodgson, 2012; Picardo et al., 2019). Moreover, the agriculture, intensive animal husbandry and urban wastewater, producing high concentration of nitrogen, phosphorus and other nutrients, together with climate change, enhance the eutrophication processes and provide the propitious conditions of harmful algal and cyanobacterial blooms – (HABs and cyano HABs), which have increased in intensity and frequency during the last years (Davis et al., 2009). Because of the chemical diversity of natural toxins, they may produce different toxic effects. The most common adverse effects include hepatotoxicity (e.g. microcystins) (Testai et al., 2016), neurotoxicity (e.g. anatoxin) (dos Santos et al., 2019), dermal toxicity (Funari and Testai, 2008) (e.g. T-2 toxins) and cytotoxicity (Froscio et al., 2009) (e.g. cylindrospermopsin) to the general inhibition of protein synthesis. The toxicity assessment and the hazard caused by natural toxins can be understood thanks to their physical and chemical properties, fate and occurrence in the environment.

Several legislations have been implemented around the world. In Europe, the principal regulations are the Water Framework Directive, the Drinking water directive (Council Directive 98/83/EC, currently under revision), and the EU Bathing Water Directive 2006/7/EC (Council directive 98/83/E, 1998; National Center for Biotechnology Information, 2020). However, these regulations are based on the analysis of target compounds, while other groups of biotoxins, such as plant toxins, are insufficiently studied and not yet regulated. Besides that, due to climate change, the occurrence of certain groups of toxins not expected in some regions will increase. As reported by Manning et al. (Manning and Nobles, 2017) human pollution and the consequent eutrophication of water bodies, will cause an unbalanced competition between cyanobacteria and other bacteria. This will allow longer HABs events with higher levels of cyanotoxins (Manning and Nobles, 2017). For these reasons, the characterisation of the complete composition of natural toxins in surface waters, including secondary metabolites and degradation products, is needed.

During the last decade, a significant number of analytical methods have been developed for the target quantification of natural toxins in freshwater environments, in particular, based on liquid chromatography coupled to mass spectrometry (LC-MS). In most cases, the analytical approaches have been developed to quantify targeted toxins or a selected group of toxins. For example, for cyanotoxins, showing in general limits of detection ranging between 0.16 ng/L and 37 μg/L (Turner et al., 2018; Gambaro et al., 2012; Yen et al., 2011; Aguete et al., 2003; Roy-Lachapelle et al., 2019).

The availability of high-resolution mass spectrometry techniques (HRMS) opened a new window for the detection of low molecular weight compounds, such as natural toxins. These techniques allow the research of unknown compounds and their tentative identification in different degrees of confidence, as previously reported by Schymansky et al. (Schymanski et al., 2014). Besides, suspect and non-target screening approaches are of particular interest in the research of emerging contaminants and those lacking analytical standards, as it is the case for a significant number of natural toxins. It should be highlighted that, in spite of the great variety of natural toxins currently identified, the information of their presence in the environment, such as in surface waters and water reservoirs, is still quite limited.

Moreover, different analytical approaches for the detection of target compounds or groups with similar chemical characteristics, have been developed. However, there is still a lack of analytical methods able to identify a wide range of natural toxins part of the total natural charge in the aquatic environment. Regarding the research of natural toxins, the lack of analytical standards is an additional issue for their identification.

This work aimed to develop a suspect screening method to establish the natural toxins profiles in natural waters. Therefore, the specific goals of this study were: (i) develop a homemade triple-stage solid-phase extraction (SPE) procedure to isolate a wide range of natural toxins from different groups and physicochemical characteristics. The good performances of the extraction procedure were tested with a selected group of natural toxins part of different classes and polarities including cyanotoxins Anatoxin (Ana-a), cylindrospermopsin (Cyn), microcystins (MCs) and their congeners -LR, -LY and -YR, nodularins (Nods); fungal toxins aflatoxin B_1 ($AflB_1$), kojic acid (Kja) and ochratoxin-A (Ot-A); and plant toxins baicalein (Bai), scopolamine (Sco), atropine (Atr), cinchonine (Chn), and B-asarone (B-as); (ii) to collect an in-house natural-toxins list, including cyanotoxins, plant toxins, and mycotoxins that are susceptible to be found in natural waters, to facilitate the screening process; (iii) to develop and validate a suspect screening approach based on high-performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-HRMS) using a Q-Exactive Orbitrap mass analyser; (iv) to validate the method for the target analysis of selected compounds; and (v) to assess the performance of the new analytical combined approach in real samples collected in the Ter River near the Barcelona water catchment area.

Despite the significant work carried out during the recent years in suspect screening approaches, to the authors' knowledge, the screening method proposed is the first one specifically designed for the comprehensive tentative identification of a wide range of natural toxins in surface waters.

2. Experimental

2.1. Chemicals and reagents

Ana-a 1 mg (99%) standard was supplied from Santa Cruz Biotechnology (Dallas, TX, USA). Cyn, 25 μg (99%) was purchased from BOCSci (BOC Sciences, Ramsey Road Shirley, NY, USA). MC-LR,

10 μg/mL; MC-YR, 10 μg/mL, MC-LY, 10 μg/mL and Nod 10 μg/mL; were >99% purity from Cyano (Cyanobiotech GmbH, Berlin, Germany). $AflB_1$, 25 μg/mL (99%) were bought from Merck (Darmstadt, Germany). Baicalein (Bai), 100 mg (98%); Scopolamine (Sco)1 mg (98%); (+)-cinchonine; (Chn) 25 g (99%); Atr, 1 g (99%); Kja 100 mg (98%); B-asa, 1 g (98%) and Ota-a 1 mg (99%) were purchased from Merck (Darmstadt, Germany) (Table S1). Methanol and acetone HPLC grade were supplied from Merck (Darmstadt, Germany) while water HPLC grade was obtained from Baker (Madrid, Spain).

2.2. Samples and sampling sites

Freshwater samples were collected in the Ter River, in central Catalonia (NE Spain), in one of the drinking water catchment areas of Barcelona Metropolitan Area to assess the performance of the proposed analytical approach. A total number of 16 samples were collected in four sampling sites Point 1 (M1) 41.986133; 2.603488, Point 2 (M2) 41.982191; 2.585539, Point 3 (M3) 41.991090; 2.570144, Point 4 (M4) 41.975693; 2.395398. Sampling was carried out twice per month during May and July 2018. May is just before the expected algal bloom and July because it is one of the months more affected in general by cyanotoxins in Spain (Carrasco et al., 2006). Besides, June, July and August are mostly dry seasons were no precipitations can cause leaching of toxins in the soil while September is considered the start of the wet season expecting highest levels of plant toxins in the water. Mycotoxins are instead produced along the entire year depending on the climate conditions. But, during summer near water bodies, the higher relative humidity, increase the production of mycotoxins (Al-Gabr et al., 2014).

The samples were collected in amber glass bottles, and before the sampling, the pH, temperature, pO_2 and conductivity were measured on-site. Samples were transported at 4 °C and then were frozen at −40 °C till the initiation of the analytical process.

2.3. Sample pre-treatment

Each sample was processed in an ultrasonic bath for 20 min at a power of 200 W and a frequency of 60 Hz to disrupt cells and release the intracellular toxins. After sonication, the samples were filtered through a glass microfiber filter GF/B grade (Sigma Aldrich, Steinheim, Germany) and toxins were isolated by off-line solid-phase extraction (SPE). A hand-made cartridge was prepared and coupled to a HLB plus cartridge to achieve the maximum recovery of toxins with different ranges of polarity. The custom SPE cartridge was prepared as follows: a 3 mL volume glass cartridge was manually filled with a double layer of 200 mg (top side) of porous graphitized carbon (PGC) 120 mesh (Sigma Aldrich, Steinheim, Germany) and 200 mg of Bond-Elut PPL (PPL) 120 mesh (Agilent, Santa Clara, CA, USA) separated by a Teflon frit. The cartridge was connected to an Oasis HLB plus (225 mg sorbent/6 mL) cartridge (Waters Corporations, Mil-ford, MA). Water samples (100 mL) were loaded through the cartridges at a flow rate of 2 mL/min previously conditioned with 10 mL of methanol and 10 mL of water, both acidified with 0.5% of formic acid (FA). After loading, the cartridges were dried under vacuum and were switched to elute the analytes in backflush. To achieve this, the PGC/PPL cartridge was reversed, while the HLB cartridge remained in the same position connected to the bottom side with the same flow direction. The toxins were eluted with 15 mL of water/methanol 2:8 (*v/v*), followed by 15 mL of methanol and 15 mL of acetone/methanol 50:50 (*v/v*). All the solvents were previously warmed at 45 °C before each elution. The eluate was evaporated almost to dryness and re-dissolved in 1 mL of mobile phase.

2.4. Liquid chromatography

The chromatograph was an Acquity high-performance liquid chromatograph system (Waters Corp, Milford, MA, USA). The chromatographic separation was achieved using a C_{18} reversed-phase column Lichrosphere, (125 mm × 2 mm i.d., 5 μm) (Merck, Barcelona, ES). A binary mobile phase was composed of water (solvent A) and acetonitrile (solvent B) both acidified with 0.1% of FA. The elution gradient was programmed as follows: From 0 to 3 min, 10% B; from 3 to 13 min, B was linearly increased to 90%; 13–15 min, stabilized at 90% B; 15–16 min B decreased linearly to 10%; 16–20 min, column stabilization with a 10% of solvent B. Extracts were kept at 8 °C in the autosampler. An aliquot of 20 μL was injected into the LC-HRMS system with a flow rate of 0.25 mL/min.

2.5. Orbitrap Q-Exactive mass spectrometry

A Thermo Scientific Orbitrap Q-Exactive mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with heated electrospray ionisation (HESI) probe was used in positive and negative mode. The optimal source parameters were as follows: spray voltage of 3.75 kV (+) and −3.25 kV (−), sheath flow gas of 20 a.u., auxiliary gas of 20 a.u., and sweep gas of 5 a.u. Heater and capillary temperature were set at 300 °C with an S-lens RF level at 60%. The acquisition was performed in full-scan-data dependant acquisition (FS-$ddMS^2$) mode with an inclusion list of 100 most probable suspect compounds. In Table 1, the acquisition parameters are summarised.

2.6. Data processing: suspect screening of natural biotoxins

Data processing was performed using the Xcalibur Qual Browser software (Thermo Fisher Scientific, San Jose, CA, USA), and Compound Discoverer software version 2.1 v. x86 (Thermo Fisher Scientific, San Jose, CA, USA), respectively.

For the suspect screening approach, first a home-made list of natural toxins was assembled collecting data from literature and other databases containing the exact mass of 2380 compounds (Table S2 of the supporting information). Besides, the information contained in two online databases, Chemspider for structural information and MzCloud, as mass spectra database was used. The workflow (Fig. 1) and the tentative identification criteria were based on those described by Krauss (Krauss et al., 2010) and Schymanski (Schymanski et al., 2014). Other filters applied were mass tolerance of 5 ppm, the retention time (tolerance ± 2.5% min) if available, isotopic fit (>90%), fragmentation and the previously mentioned parameters in addition to the mass peak resolution and

Table 1
Acquisition parameters of the reported MS methods.

	Acquisition parameters
Method duration (min)	20
Acquisition mode	Full MS/$ddMS^2$
Polarity	(+); (−)
Resolution (FWHD)	35,000 FS 17,500 $ddMS^2$
AGC target	1.00E+05
Max IT	50
Scan Range (*m/z*)	75–1100
Spectrum data type	Centroid
Loop count	3
Top-N	3
Isolation window (*m/z*)	2
Collision Energy (CE)	35 (All toxins) 70 (Aflatoxins)

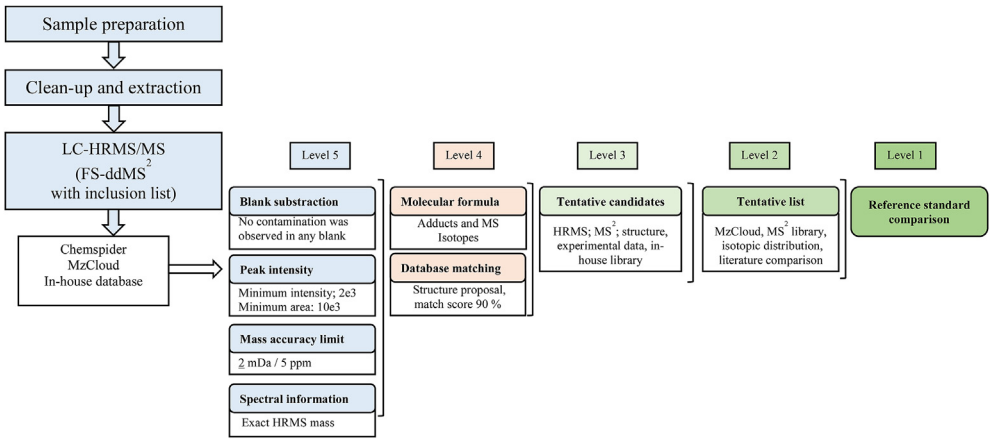

Fig. 1. Tentative identification workflow inspired by Schymansky et al. (Schymanski et al., 2014).

response. The suspect screening was performed as follows; the FS-ddMS^2 spectra were processed with Compound Discoverer that helped to obtain a list of candidates between the ones contained in the inclusion list. These suspect natural toxins were finally checked and confirmed to level 2 matching the experimental fragmentation spectra and comparing them with theoretical patterns encountered in the literature. Finally, coincident compounds with the available standards were confirmed, identification at level 1.

2.7. Quality assurance

The proposed suspect screening was tested using spiked samples with 14 representative natural toxins pertaining to different groups and showing different physicochemical properties; Plant toxins (Bai, Sco, Atr, Chn, B-asa); Cyanotoxins (Ana-a, Cyn, MC-LR, MC-LY, Nod and MC-YR); Fungal toxins (Ota-a, Kja, AflB$_1$). First, the extraction performance was assessed with the spiked samples. Then, the samples were processed to evaluate the suspect screening approach. Finally, the analytical parameters of the entire method for target analysis of selected toxins were assessed, and the recoveries, method limits of detection (MDL), quantification (MQL), selectivity, linearity, and precision were determined.

2.7.1. Spiking experiments

Fortified samples were prepared in ultra-pure water and artificial freshwater (AFW). AFW was prepared according to the description of Lipschitz and Michel (2002), the organic matter was simulated by adding 10 mg/L of humic acid of technical grade from Sigma-Aldrich (reference 53,680), and the pH was adjusted to 6.5 with formic acid 1.0 M.

In both matrices, the spiked samples were prepared by fortification at seven different concentrations between 0.5 and 100 μg/L, using the 14 representative toxins. The spiked samples were left for 1 h at room temperature to ensure proper distribution in the matrix. Besides, three pristine natural samples, free of the representative selected toxins, were additionally spiked at the same concentrations following the same procedure. Samples were processed as reported in section 2.3 working in triplicates adding three procedural blanks that showed no contamination for the entire method. However, procedural blanks were performed during each analysis and the signals in the blanks were quantified and subtracted from the signals of samples. To assess the good extraction performance, the spiked samples were compared with the corresponding calibration curve prepared in the initial composition of the mobile phase according to the elution gradient of the chromatography. In the Supporting Information section, the SPE optimization results are presented.

2.7.2. Validation of the suspect screening approach

First, the extraction procedure was evaluated in front of the selected natural toxins, and the recoveries for the representative compounds were calculated. Then, the suspect screening approach was tested using the Compound Discoverer and the data bases using the same approach as reported in 2.6.

2.7.3. Evaluation of the analytical approach for target analysis of the selected natural toxins

The linearity range, intra-assay precision, accuracy, matrix effects, the limit of detection (LOD) and limits of quantification (LOQ) for the 14 selected toxins was carried out according to the EURACHEM guidelines (Magnusson and Örnemark, 2014). The instrumental limits of detection (iLOD) were calculated by progressive dilution to the lowest concentration where each compound could be detected. Instrumental linearity and sensitivity were estimated as the squared Pearson index (R^2) and the slopes of the calibration curves, respectively. Instrumental reproducibility (inter-day precision) was calculated as the average percentage of the relative standard deviation (RSD %) of standard solutions (six replicates) at seven concentration levels on three consecutive days.

The MDL and MQL were based on matrix-matched calibration curve points. MDL of each analyte was defined as the lowest concentration for which the peak area was, at least, three times the signal-to-noise, while the MQLs were established as the lowest concentrations which fulfilled the criteria: signal-to-noise ratio, at least, 10; relative standard deviation of three replicates, below 19%; Gaussian peak shapes; less than 5 ppm of exact mass error; and isotopic pattern similarity.

Recoveries and precision were assessed by repeating six times the procedure at a concentration of 0.5 μg/L during three different days. Precision expressed as intraday and inter-day repeatability was calculated repeating the procedure in the same day and during a period of 3 months (2 times per week), respectively.

3. Results and discussion

3.1. Sample preparation for a wide range of natural toxins in water

An SPE procedure was developed to extract wide polarity range of natural toxins from water samples. Based on previously reported results four different sorbents were tested: Oasis HLB Plus (Oasis-

HLB), from Waters (Waters Corp, Milford, MA, USA), ISOLUTE®C_{18} (C_{18}) from Biotage (Biotage AB, Uppsala, Sweden), EnviCarb polygraphitized carbon (PGC) from Sigma Aldrich (Steinheim, Germany), and Bond-Elut PPL from Agilent (Santa Clara, CA, USA).

Oasis HLB is a polymeric reversed-phase sorbent composed by a copolymer of divinylbenzene and vinyl pyrrolidinone that has been efficiently used for the enrichment of natural toxins from environmental samples (Schenzel et al., 2010). Good recoveries were obtained for polar to moderately polar toxins as expected such as aflatoxins, nodularin and microcystins in general, except for MC-LF (recoveries < 10%) because it is a non-polar compound. C_{18} is a hydrophobic silica bonded sorbent efficient to isolate non-polar and moderately polar compounds from aqueous matrices. Some studies reported the high recovery of natural toxins when it was combined with other phases such as PGC (Liu and Scott, 2011). Here, C_{18} showed good recoveries for polar to moderately polar compounds as well such as microcystins but with lower recoveries than Oasis HLB. Kja, Sco, Atr, Ana-a, Chn, Cyn, were well retained with the PGC (24–58%). The Bond Elut PPL is a styrene-divinylbenzene (SDVB) polymer that has been modified with a non-polar surface and is efficient in extracting dissolved organic matter (Dąbrowska et al., 2003; Raeke et al., 2016; Goss et al., 2017). As expected, this sorbent alone was not suitable for the retention of natural toxins but combined with other phases it improved the recoveries thanks to the lower content in organic matter into the final sample and to the diminished matrix effects.

With these results, a homemade triple-stage SPE method was developed and optimised, consisting of PGC-PPL-HLB in series. This combination showed the best recoveries, as can be shown in Fig. S1 of the Supporting Information. The correspondent K_{ow} have been also reported as supplementary information in Table S1.

Elution solvents were as well optimised. In Fig. 2, the recoveries obtained with different solvents or mixtures in a single step are summarised. The best elution was achieved with water: methanol 8:2 (v/v) which eluted the most of the natural toxins especially MC-LR, Kja, Sco, Ana-a, Atr, Bai and Cyl with recoveries ranging between 24 and 79%. The final volume was set at 15 mL to ensure a better recovery of toxins. Then, different mixtures were tested to improve the recovery from different sorbents. A mix of methanol/acetone 1:1 (v/v) was selected to ensure a better contact with the most polar phase (PGC). As can be seen in Fig. S1A, the recoveries were between 2.4 and 53%.

Finally, the best elution sequence was shown to be 15 mL of water: methanol 8:2 (v/v) followed by 15 mL of methanol/acetone 1:1 (v/v) and 15 mL more of methanol 100%.

The influence of the pH of elution solvents was studied as well. pH 2 showed in general, low recovery rates, basic pH (pH 11) gave satisfactory rates for Ana-a, MC-LR and MC-YR, but pH 7 yield the best compromise between loading efficiency and compound neutrality **(Fig. S1B).** Elution temperature (T°) was studied. The strongest elution was obtained working with warm solvents at 40 °C. The optimal approach gave recovery rates between 53 and 98% for the selected group of natural toxins of different polarities.

3.2. Suspect screening

Tentative structural assignments were based on the criteria previously described by Schymanski et al. (2015). From the suspect list, one hundred compounds were selected for the inclusion list considering the botanical diversity of the area, the most probable species present and the climatic conditions that can determine the production of certain natural toxins. This limit was decided as a compromise of the analytical time and the scan rate of the QExactive. A first attempt with the entire suspect list (2377 precursor ions) resulted in a very poor fragmentation spectrum with less than one scan for each peak which are not enough for the identification purposes of this step. QExactive data scan speed at a resolution of 17,500 can reach 12.5 Hz with an injection time of 50 ms in a range of 75–1100 *m/z*, but it required a higher analytical time to process the whole number of entries. Finally, a lower number of entries

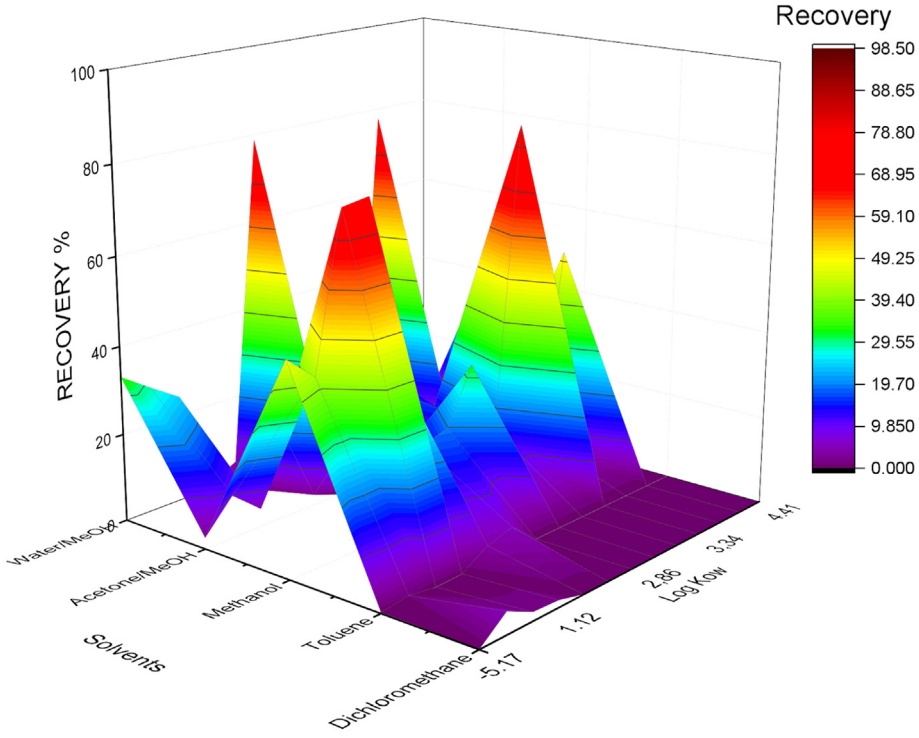

Fig. 2. Recoveries obtained using different solvents during the elution step of the SPE.

provided a more precise mass spectrum and at least three scans per peak, enough for identification purposes and it has then been used for the entire experiments. After pre-identification using Compound Discoverer, the fragmentation of the tentatively selected compounds was compared with known fragmentation patterns collected in on-line libraries such as MzCloud and MassBank.

The lowest levels of identification confidence (level 5, 4 and 3) comes from the similarity between experimental and the exact mass typical fragments and adducts proposed by the online databases. (<5 ppm). The probable structures were established matching the MS^2 spectrums obtained with the FS-ddMS^2 mode and the spectrums reported in the literature, considering the same transitions and the same ratio of the product-ions (level 2). This is a high level of confidence but cannot be confirmed since it can lead to false positives. Finally, only those compared with standards and with coincident retention time compared at a tolerance of ±2.5% were confirmed to level 1 (Fig. 1).

These criteria were applied to tentatively identify natural toxins in 16 samples collected from four different sampling sites along the Ter River near the water catchment area of Barcelona city. In Fig. 3, an example of tentative identification in a real sample is presented. Following this procedure, a range between 14 and 25 structures have been tentatively identified in each point. In the case of MCs and Nod, the typical ADDA fragment (135.0505 *m/z*) was used to establish as tentative candidate. The ADDA fragment is a highly stable product ion originated by the typical moiety corresponding to the [phenyl-$CH_2CH(OCH_3)^+$ ion contained in MCs and Nod, that has been considered in various analytical methods for the identification of MCs and Nod using LC-*ESI*-MS/MS (Mbukwa et al., 2012; Hummert et al., 2001).

Finally, 23 natural toxins candidates were tentatively identified with a confidence level 2 (Table 2).

Most of them were frequently reported toxins except two of them aspionene, and laudanosine, which have been few times reported (Lehner et al., 2011; Fodale and Santamaria, 2002). It should be highlighted that a wide variety of toxins tentatively identified were phytotoxins (12), followed by mycotoxins (8).

The 3-acetoxytropane and hygrine are tropane alkaloids found in Datura stramonium of the Solanaceae family. Their alkaloids produce psychoactive effects and eventually, death (Boopathi, 2019). On the other hands, phytoestrogens such as coumestans exhibit estrogenic activity with reproductive effects on cattle. The presence of both groups of compounds tentatively identified in the Ter river was not surprising. Datura stramonium is extensively present along the entire region of Catalonia (Ministerio para la transición ecológica y el reto demografico, 2020), and its presence can be related to the leaching from the aerial parts of the plants into the water. Allantoin, geranylacetone and 20-deoxyingenol are as well plant secondary metabolites that were tentatively identified in some of the samples, and their production increases in the response of stress periods enhancing seedling tolerance (Takagi et al., 2016). Another compound that was tentatively identified in the samples was, xanthotoxol, which is the most abundant toxin from *Cnidium monnieri*. The abundance of *Cnidium monnieri* in Catalonia can be related to the presence of suspect xanthotoxol in Ter River. Moreover, there are different plants endemic of this area that can produce this metabolite. On the other hand, the chemical properties of xanthotoxol allow a quick transfer from plants to soils

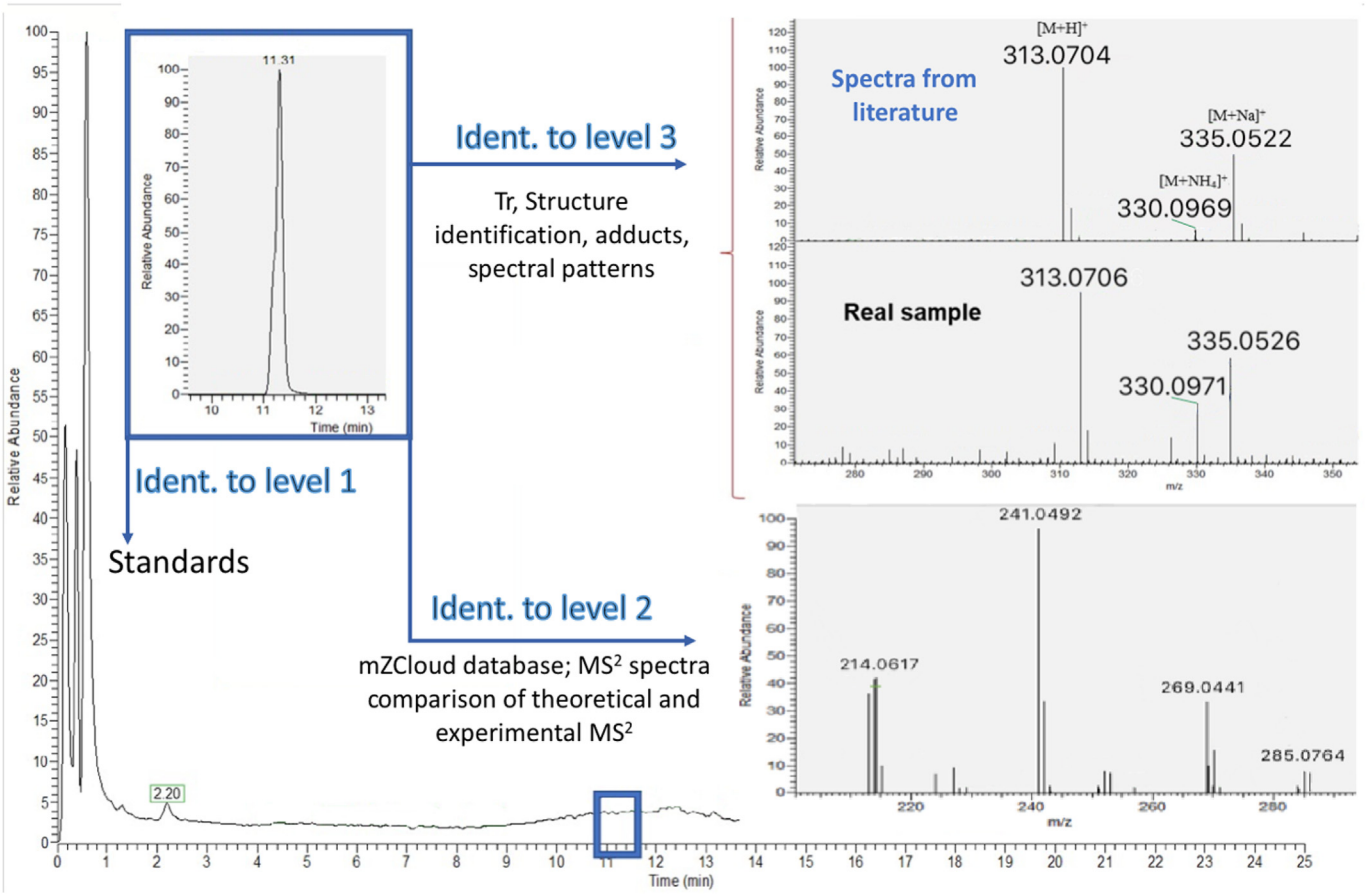

Fig. 3. Example of tentative identification of aflatoxin B_1.

Table 2
Structures tentatively identified, chemical and features for their identification.

Compound Name	Chemical formula	Ret. Time [min]	Most int. ion species [m/z]	Ion specie	Theoretical [M+H]+	Observed [M+H]+	Rel. mass dev. [ppm]	m/z found	Ion specie	MS2	Ion specie
3-Acetoxytropane	$C_{10}H_{17}NO_2$	9.1	206.1151	$[M+Na]^+$	184.1334	184.1332	−1.1	184.1332	$[M+H]^+$	125.12062 146.09452	$[M+H-CH3COOH]^+$ $[M+Na-CH3COOH]^+$
20-Deoxyingenol	$C_{20}H_{28}O_4$	13.2	301.1396	$[M+H-CH_3OH]^+$	333.2060	333.2046	−4.3	355.1879	$[M+Na]^+$	315.1953	$[M+H-H_2O]^+$
Aflatoxin B_2	$C_{17}H_{14}O_6$	11.2	315.0863	$[M+H]^+$	315.0863	315.0863	0.0	337.0682	$[M+Na]^+$	297.2076 271.1543	$[M+H-H_2O]^+$ $[M+H-CO_2]^+$
Aflatoxin G_1	$C_{17}H_{12}O_7$	11.0	329.0655	$[M+H]^+$	329.0656	329.0655	−0.2	367.1270	$[M+K]^+$	311.1077 312.1309 285.0643	$[M+H-H2O]^+$ $[M+H-OH]^+$ $[M+H-CO_2]^+$
Aflatoxin G_2	$C_{17}H_{14}O_7$	10.7	331.0812	$[M+H]^+$	331.0812	331.0812	0.0	353.0631	$[M+Na]^+$	313.0898 287.1463 286.1130	$[M+H-H_2O]^+$ $[M+H-CO_2]^+$ $[M+H-COOH]^+$
Aflatoxin B_1	$C_{17}H_{12}O_6$	11.3	313.0707	$[M+H]^+$	313.0707	313.0707	0.1	335.0526 351.0266	$[M+Na]^+$ $[M+K]^+$	253.0495 269.08138 241.0477	$[M+H-CH_3COOH]^+$ $[M+H-CO_2]^+$ $[M+H-C_2H_5COCH_2]^+$
Allantoin	$C_4H_6N_4O_3$	0.4	159.0506	$[M+H]^+$	159.0513	159.0506	−4.2	197.0158 181.0063	$[M+K]^+$ $[M+Na]^+$	144.0050 111.0235	$[M+H-NH_3]^+$ $[M+H-CHOHNH_2]^+$
Anatoxin-a	$C_{10}H_{15}NO$	0.7	166.1226	$[M+H]^+$	166.1223	166.1226	1.8	204.1032 188.0488	$[M+K]^+$ $[M+Na]^+$	123.1048 151.0997	$[M+H-CH_3CO]^+$ $[M+H-CH3]^+$
Arecoline	$C_8H_{13}NO_2$	1.9	156.1019	$[M+H]^+$	156.1019	156.1019	0.0	194.0582 178.0838	$[M+K]^+$ $[M+Na]^+$	141.0789 96.08132	$[M+H-CH_3]^+$ $[M+H-CH_3OOH]^+$
(−)-Aspidospermine	$C_{22}H_{30}N_2O_2$	12.5	355.2380	$[M+H]^+$	355.2380	355.2380	0.0	377.2199 393.1940	$[M+Na]_+$ $[M+K]_+$	337.1187 322.1774	$[M+H-H_2O]^+$ $[M+H-CH_3OH]^+$
Aspionene	$C_9H_{16}O_4$	11.0	187.0976	$[M-H]^-$	187.0976	187.0976	0.0	209.0751 231.9252	$[M-H+CH_3OH]^-$ $[M-H+HCOOH]^-$	142.0953 168.9833	$[M-H-COOH]^-$ $[M-H-H_2O]^-$
Azelaic Acid	$C_9H_{16}O_4$	11.0	189.1122	$[M+H]^+$	189.1121	189.1122	0.1	227.0680 211.0941	$[M+K]_+$ $[M+Na]_+$	172.10994 144.11503	$[M+H-OH]^+$ $[M+H-COOH]^+$
Coumestan	$C_{15}H_8O_3$	0.2	237.0546	$[M+H]^+$	237.0546	237.0546	0.1	259.0471 274.8998 254.0404 268.8908 281.0709	$[M+Na]_+$ $[M+K]_+$ $[M+NH_4]_+$ $[M+CH_3OH]^+$ $[M+FA]^+$	219.0446 220.9143	$[M+H-H_2O]^+$ $[M+H-OH]^+$
Cryptolepine	$C_{16}H_{12}N_2$	0.4	243.1064	$[M+K]^+$	233.0700	233.0701	0.5	246.1253 263.1173 255.0740	$[M+CH_3CN+H]^+$ $[M+CH_3COOH-H]^+$ $[M+Na]^+$	218.0938	$[M+H-CH_3]^+$
Embelin	$C_{17}H_{26}O_4$	12.5	295.1904	$[M+H]^+$	295.1904	295.1904	0.0	317.1723 333.1459	$[M+Na]^+$ $[M+K]^+$	278.1174	$[M+H-OH]^+$
Geranylacetone	$C_{13}H_{22}O$	0.1	217.0174	$[M+Na]^+$	195.1743	195.1743	0.0	233.1541	$[M+K]^+$	177.1643 178.0937	$[M+H-H_2O]^+$ $[M+H-OH]^+$
Hygrine	$C_8H_{15}NO$	2.18	142.1222	$[M+H]^+$	142.1226	142.1222	−2.8	142.1222 180.1015	$[M+H]^+$ $[M+K]^+$	126.0909 111.0387 84.0805	$[M+H-CH_3]^+$ $[M+H-CH_2OH]^+$ $[M+H-C_3H_6O]^+$
Lantadene D	$C_{34}H_{52}O_5$	12.7	579.3507	$[M+K]^+$	541.3888	541.3879	−1.7	563.3225	$[M+Na]^+$	468.3190 439.091 343.2533	$[M+H-C_4H_8O_2]^+$ $[M+H-C_{10}H_{14}O_2N]^+$

(*continued on next page*)

Table 2 (*continued*)

Compound Name	Chemical formula	Ret. Time [min]	Most int. ion species [m/z]	Ion specie	Theoretical [M+H]+	Observed [M+H]+	Rel. mass dev. [ppm]	m/z found	Ion specie	MS2	Ion specie
Laudanosine	$C_{21}H_{27}NO_4$	13.2	325.2201	$[M+H-CH_3OH]^+$	358.2013	358.2010	−0.8	390.2423	$[M+H+CH_3OH]^+$	206.1175 189.091 151.0754	$[M+H-C_{13}H_{24}O]^+$ $[M+H-C_9H_{12}O_2]^+$ $[M+H-C_{10}H_{14}O_2N]^+$
Microcystin-LR	$C_{49}H_{74}N_{10}O_{12}$	12.0	995.5560	$[M+H]^+$	995.5560	995.5560	0.0	498.7860	$[M+H]^{2+}$	135.0440 931.4331	$[M+H-ADDA]^+$ $[M+H-CN_3H_4]^+$
Nodularin	$C_{41}H_{60}N_8O_{10}$	11.7	825.4505	$[M+H]^+$	825.4505	825.4505	0.0	848.6317 466.2278	$[M+Na]^+$ $[M+H]^{2+}$	807.4016 764.3502	$[M+H-H_2O]^+$ $[M+H-CN_3H_4]^+$
Stipitatic acid	$C_8H_6O_5$	0.4	183.0269	$[M+H]^+$	183.0288	183.0289	0.5	205.0369 221.0000	$[M+Na]^+$ $[M+K]^+$	137.9976 166.9685 204.0627	$[M+H-COOH]^+$ $[M+H-OH]^+$ $[M+Na-OH]^+$
Xanthotoxol	$C_{11}H_6O_4$	0.4	225.0103	$[M+Na]^+$	203.0379	203.0399	9.9	240.9447 261.0761	$[M+K]^+$ $[M+CH_3COOH-H]^+$	184.8984 157.0714	$[M+H-H_2O]^+$ $[M+H-CHOOH]^+$

and water (Real et al., 2019; Moreno-Pedraza et al., 2019). However, no quantification was possible due to the lack of standards, and levels are only relatives to the intensities.

In Fig. 4, the frequency of the different groups of toxins tentatively identified in each point is reported. Also, in Table 3, the potential structures in each point and sampling month are summarised. M1 corresponds to the location near the Barcelona water catchment area which is downstream respect to the other sampling sites. Due to their characteristics of temperature, sun exposition, low water flow, and vegetation, it presented the major variety of toxins, as expected. In the same manner, M4 is located upstream and was the point with a lower occurrence of natural

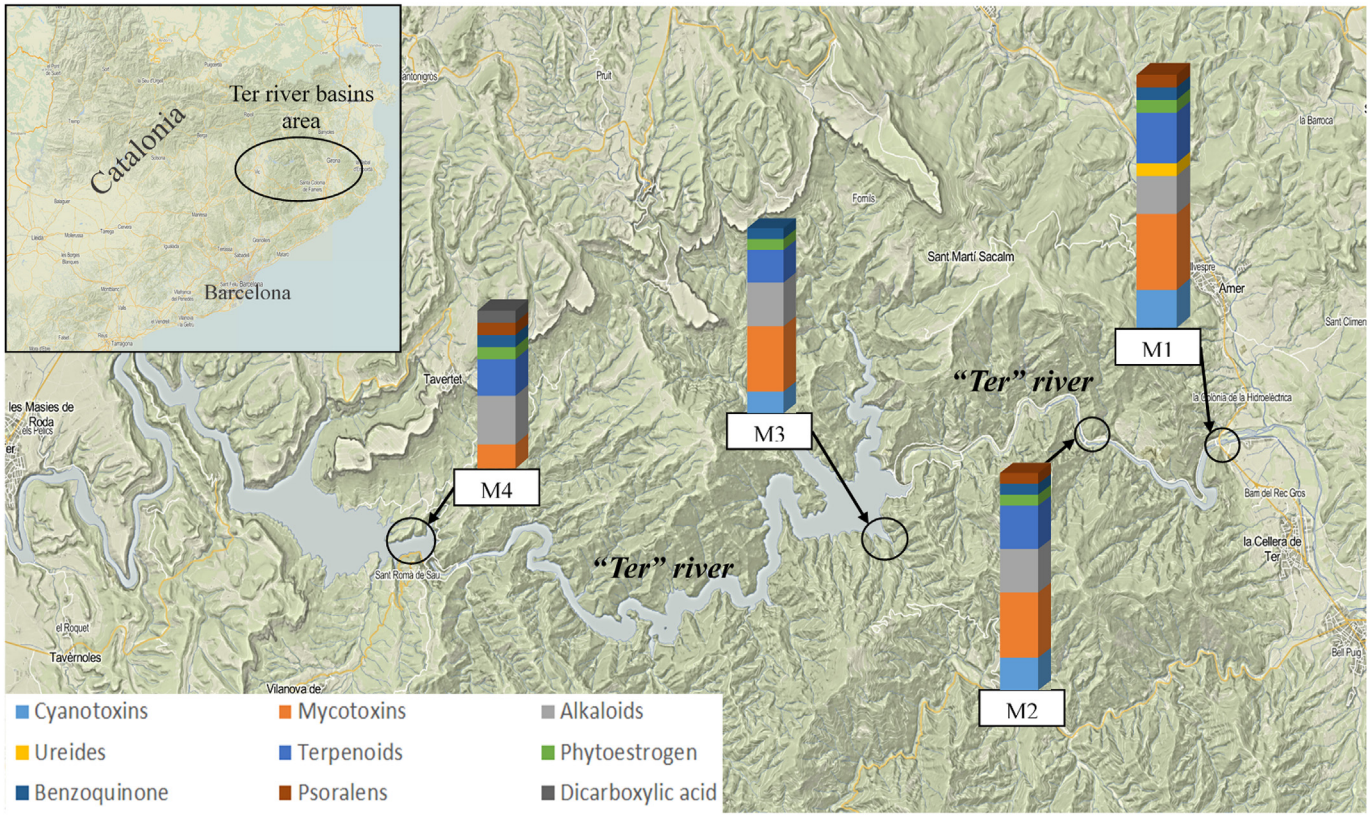

Fig. 4. Natural toxins screening results along the four points of the Ter river in Catalonia, Spain (Sampling points M1, M2, M3, M4).

Table 3
List of natural toxins tentatively identified in four sampling points during May and July; areas encountered between 10^4 and 10^6 a.u., quantification was not possible due of the lack of standards.

Sampling points	MAY				JULY			
	M1	M2	M3	M4	M1	M2	M3	M4
Natural toxins	***Mycotoxins***							
Aflatoxin B_1	X			X	X	X	X	
Aflatoxin B_2					X			
Aflatoxin G_1					X			
Aflatoxin G_2					X			
Allantoin					X	X	X	X
Aspionene							X	
Stipitatic acid			X	X	X			
Kojic acid	X	X	X	X	X		X	X
Azelaic Acid				X	X			
	Phytotoxins							
Embelin								X
Buddledin B	X		X					X
Xanthotoxol	X	X	X	X	X	X	X	X
Arecoline						X	X	X
(−)-Aspidospermine	X	X	X	X	X	X	X	X
20-Deoxyingenol		X	X		X		X	
3-Acetoxytropane	X	X	X			X		X
Coumesta/ coumetstrol	X	X			X	X	X	
Geranylacetone					X	X	X	
Lantadene D			X		X			
Cryptolepine				X				X
Laudanosine	X		X	X		X	X	X
B-Asarone	X	X	X	X	X	X	X	X
Hygrine			X			X		
	Cyanotoxins							
Anatoxin-a	X				X	X		
Microcystin-LR	X				X	X		
Nodularin	X				X	X	X	

toxins tentatively identified.

Downstream, an increasing presence of natural toxins can be observed especially in July.

Comparing the results of May and July, mycotoxins and cyanotoxins presented a significant increase on the number of structures tentatively identified, while in the case of phytotoxins the number of compounds in July was only slightly higher than during May. Especially in the case of cyanotoxins, a significant increase was observed in July in the case of MC-LR and Nod.

Very few information is available on the growth of fungal species, especially in water environments.

Fungal species that naturally grow in streams tend to decay and to be adsorbed into plants. From the plants, bacteria and mycotoxins can leach into water streams leading to the contamination of surface water used for drinking water production. Moreover, mycotoxins can grow in surface water, groundwater and into the drinking water supply system (Al-Gabr et al., 2014; Hageskal et al., 2006; Magwaza et al., 2017).

During July and May, Catalonian's climate becomes warmer and humid, with more sun exposition with a consequent increase of water temperatures. During May and July 2017, the average temperature of inland Catalonia was above the 26 °C. with low precipitations (Servei Meteorològic de Catalunya). These factors aimed at the onset of the cyanobacterial algal bloom and also created the optimal conditions to the growth of moulds and fungi. The production of cyanotoxins and mycotoxins is independent, and to the author's knowledge, no article reported a dependence or correlation with the production of mycotoxins and cyanotoxins.

Finally, $AflB_1$, Ana-a, Atr, Bai, B-Asa, Chn, Kja, Nod and MC-LR were confirmed by standards and quantified.

3.3. Target analysis and quantification

The analytical procedure based on SPE-HPLC-HRMS employed for the suspect screening of natural toxins was validated for a selected group of toxins that were as well used for the spiking experiments. The recoveries obtained were in a range between 53 and 96%. MDLs ranges were between 0.07 and 1.22 μg/L while MQLs were between 0.02 and 4 μg/L. In Table S1 of the supporting information, the analytical parameters are summarised. The results of the 16 analysed samples reported 26 natural toxins tentatively identified in these natural waters (Table 3). Nine of them were confirmed and quantified (Table 4). As reported in Table 4, only Ana-a MC-LR and Nod were above the MQL in the sampling points M1, M2 and M3. MC-LR is the only toxin which has been regulated (Council directive 98/83/E, 1998) with a limit of 1 μg/L, and as can be seen in none of the samples, this limit was exceeded.

4. Conclusions

HRMS showed versatility and potential to carry out a suspect screening of natural toxins in water reservoirs and recreational waters. Suspect screening could be used as a routine tool to perform a comprehensive assessment of natural toxins in water reservoirs and recreational waters. The clean-up approach using a homemade triple-stage SPE was shown efficient for a wide range of polarities with recovery rated between 53 and 96% and showed good MQLs, between 0.02 and 4 μg/L, for the selected toxins using external calibration.

The suspect screening approach was carried out in different sampling sites close to one of the Barcelona catchment sites. The results showed a good performance of the new approach. One of the main contributions of this study is that 23 natural toxins were tentatively identified, indicating the presence of plant toxins and mycotoxins that in general are not monitored in surface waters but they can contribute to the final toxicity. Finally, 9 toxins ($AflaB_1$,

Table 4
Concentrations of the natural toxins confirmed with standards.

	MAY 1st Sampling				**MAY 2nd Sampling**			
	M1	**M2**	**M3**	**M4**	**M1**	**M2**	**M3**	**M4**
Toxin name	μg/L				μg/L			
$AflaB_1$	<MDL	<MDL	<MDL	<MDL	0.36	<MDL	<MDL	0.47
Ana-a	<MDL	<MDL	<MDL	<MDL	0.21	<MDL	<MDL	<MDL
Atr	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Bai	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
B-Asa	3.1	2.2	0.8	0.6	2.8	1.5	1.2	0.2
Chn	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Kja	2.3	0.3	4.1	3.2	2.5	<MDL	3.8	2.6
Nod	<MDL	<MDL	<MDL	<MDL	0.25	<MDL	<MDL	<MDL
MC-LR	<MDL	<MDL	<MDL	<MDL	0.50	<MDL	<MDL	<MDL
	JULY 1st Sampling				**JULY 2nd Sampling**			
	M1	**M2**	**M3**	**M4**	**M1**	**M2**	**M3**	**M4**
Toxin name	μg/L				μg/L			
$AflaB_1$	<MDL	0.27	0.36	<MDL	0.26	0.27	0.39	<MDL
Ana-a	0.73	0.55	<MDL	<MDL	0.60	<MDL	<MDL	<MDL
Atr	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Bai	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
B-Asa	2.3	1.3	<MDL	4.3	1.2	1.0	0.4	3.8
Chn	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Kja	0.9	1.5	0.6	0.7	<MDL	1.2	0.8	1.2
Nod	0.91	0.49	0.4	<MDL	0.85	0.45	0.2	<MDL
MC-LR	0.24	<MDL	<MDL	<MDL	0.23	0.24	<MDL	<MDL

$AflaB_1$; aflatoxin B_1, *Ana-a*: anatoxin-a, *Atr:* atropine, *Bai*: baicalein, *B-Asa*, b-asarone, *Chn*: cinchonine, *Kja*: kojic acid, *MC-LR*: Microcystin-LR, *Nod:* nodularin.

Ana-a, Atr, Bai, B-Asa, Chn, Kja, Nod, MC-LR) were confirmed. It should be highlighted that MC-LR, the only natural toxin under regulation in natural waters, was present in 2 of the 4 sampling sites here reported, but cyanotoxins concentration did not exceed the maximum level of 1 μg/L set by the EU legislation.

Credit author statement

Massimo Picardo: Investigation, Formal analysis and writing-Original draft. Josep Sanchís: Methodology and Writing - Review & Editing. Oscar Núñez:Supervision and Writing - Review & Editing. Marinella Farré: Conceptualization,Methodology, Supervision and Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.127888.

References

Aguete, E.C., Gago-Martínez, A., Leão, J.M., Rodríguez-Vázquez, J.A., Menàrd, C., Lawrence, J.F., 2003. HPLC and HPCE analysis of microcystins RR, LR and YR present in cyanobacteria and water by using immunoaffinity extraction. Talanta *59*, 697–705. https://doi.org/10.1016/S0039-9140(02)00610–0.

Al-Gabr, H.M., Zheng, T., Yu, X., 2014. Fungi contamination of drinking water. In: Whitacre, D.M. (Ed.), Reviews of Environmental Contamination and Toxicology Volume 228. Springer International Publishing: Cham, pp. 121–139. https://doi.org/10.1007/978-3-319-01619-1_6.

Carrasco, D., Moreno, E., Sanchis, D., Wörmer, L., Paniagua, T., Del Cueto, A., Quesada, A., 2006. Cyanobacterial abundance and microcystin occurrence in Mediterranean water reservoirs in Central Spain: microcystins in the Madrid area. Eur. J. Phycol. 41, 281–291. https://doi.org/10.1080/09670260600801724.

Clucas, B., 2010. Defensive chemicals. In: Breed, M.D., Moore, J. (Eds.), Encyclopedia of Animal Behavior. Academic Press: Oxford, pp. 481–486. https://doi.org/10.1016/B978-0-08-045337-8.00293-Xpp.

Council directive 98/83/EC on the quality of water intended for human consumption. Off. J. Eur. Commun. L330, 1998, 32–54. Official Journal of European Community, L330 (1998) 32–54.

Davis, T., Berry, D., Boyer, G., Gobler, C., 2009. The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of microcystis during cyanobacteria blooms, 8, 715–725.

dos Santos, D.S., Rosa, M.E., Zanatta, A.P., Oliveira, R.S., de Almeida, C.G.M., Leal, A.P., Sanz, M., Fernandes, K.A., de Souza, V.Q., de Assis, D.R., et al., 2019. Neurotoxic effects of sublethal concentrations of cyanobacterial extract containing anatoxin-a(s) on Nauphoeta cinerea cockroaches. Ecotoxicol. Environ. Saf. *171*, 138–145. https://doi.org/10.1016/j.ecoenv.2018.12.068.

Dąbrowska, H., Dąbrowski, Ł., Biziuk, M., Gaca, J., Namieśnik, J., 2003. Solid-phase extraction clean-up of soil and sediment extracts for the determination of various types of pollutants in a single run. J. Chromatogr. A *1003*, 29–42. https://doi.org/10.1016/S0021-9673(03)00849–5.

Fodale, V., Santamaria, L.B., 2002. Laudanosine, an atracurium and cisatracurium metabolite. Eur. J. Anaesthesiol. 19, 466–473. https://doi.org/10.1017/s0265021502000777.

Froscio, S.M., Fanok, S., Humpage, A.R., 2009. Cytotoxicity screening for the cyanobacterial toxin cylindrospermopsin. J. Toxicol. Environ. Health, Part A 72, 345–349. https://doi.org/10.1080/15287390802529906.

Funari, E., Testai, E., 2008. Human health risk assessment related to cyanotoxins exposure. Crit. Rev. Toxicol. 38, 97–125. https://doi.org/10.1080/10408440701749454.

Gambaro, A., Barbaro, E., Zangrando, R., Barbante, C., 2012. Simultaneous quantification of microcystins and nodularin in aerosol samples using high-performance liquid chromatography/negative electrospray ionization tandem mass spectrometry. RCM (Rapid Commun. Mass Spectrom.) 26, 1497–1506. https://doi.org/10.1002/rcm.6246.

Goss, C.D., Wiens, R., Gorczyca, B., Gough, K.M., 2017. Comparison of three solid phase extraction sorbents for the isolation of THM precursors from manitoban surface waters. Chemosphere *168*, 917–924. https://doi.org/10.1016/j.chemosphere.2016.10.118.

Hageskal, G., Knutsen, A.K., Gaustad, P., de Hoog, G.S., Skaar, I., 2006. Diversity and significance of mold species in Norwegian drinking water. J. Appl. Environ. Microbiol. 72, 7586–7593. https://doi.org/10.1128/AEM.01628-06.

Hodgson, E., 2012. Chapter fourteen - toxins and venoms. In: Hodgson, E. (Ed.), Progress in Molecular Biology and Translational Science, vol. 112. Academic Press, pp. 373–415.

Hummert, C., Dahlmann, J., Reinhardt, K., Dang, H.P.H., Dang, D.K., Luckas, B., 2001. Liquid chromatography-mass spectrometry identification of microcystins inMicrocystis aeruginosa strain from lake Thanh Cong, Hanoi. Vietnam. Chromatographia 54, 569–575. https://doi.org/10.1007/bf02492180.

Krauss, M., Singer, H., Hollender, J., 2010. LC–high resolution MS in environmental analysis: from target screening to the identification of unknowns. Anal. Bioanal. Chem. 397, 943–951. https://doi.org/10.1007/s00216-010-3608-9.

Lehner, S.M., Neumann, N.K., Sulyok, M., Lemmens, M., Krska, R., Schuhmacher, R., 2011. Evaluation of LC-high-resolution FT-Orbitrap MS for the quantification of selected mycotoxins and the simultaneous screening of fungal metabolites in food. Food Addit. Contam. Part A, Chemistry, analysis, control, exposure & risk assessment 28, 1457–1468. https://doi.org/10.1080/19440049.2011.599340.

Lipschitz, D.L., Michel, W.C., 2002. Amino acid odorants stimulate microvillar sensory neurons. Chem. Senses 27, 277–286.

Liu, H., Scott, P.M., 2011. Determination of the cyanobacterial toxin cylindrospermopsin in algal food supplements. Food Addit. Contam. 28, 786–790. https://doi.org/10.1080/19440049.2010.501824.

Magnusson, B., Örnemark, U., 2014. Eurachem Guide: the Fitness for Purpose of Analytical Methods - a Laboratory Guide to Method Validation and Related Topics, second ed.

Magwaza, N.M., Nxumalo, E.N., Mamba, B.B., Msagati, T.A.M., 2017. The occurrence and diversity of waterborne fungi in african aquatic systems: their impact on water quality and human health, 14, 546.

Manning, S.R., Nobles, D.R., 2017. Impact of global warming on water toxicity: cyanotoxins. Curr. Opin. Food. Sci. *18*, 14–20 doi:10.1016/j.cofs.2017.09.013.

Mbukwa, E.A., Msagati, T.A.M., Mamba, B.B., 2012. Quantitative variations of intracellular microcystin-LR, -RR and -YR in samples collected from four locations in Hartbeespoort Dam in North west province (South Africa) during the 2010/2011 summer season. Int. J. Environ. Res. Publ. Health 9, 3484–3505. https://doi.org/10.3390/ijerph9103484.

Boopathi, C.A., 2019. Medicinal & Poisonous Plants of India. MJP Publisher.

Ministerio para la transición ecológica y el reto demografico, Atlas de las Plantas Alóctonas Invasoras en España. Availabe online: https://www.miteco.gob.es/es/biodiversidad/temas/inventarios-nacionales/datura_stramonium_tcm30-70093.pdf (accessed on 28 April 2020).

Moreno-Pedraza, A., Gabriel, J., Treutler, H., Winkler, R., Vergara, F., 2019. Effects of water availability in the soil on tropane alkaloid production in cultivated Datura stramonium. Metabolites 9. https://doi.org/10.3390/metabo9070131.

National Center for Biotechnology Information. PubChem database. Nodularin, CID=14217092, https://pubchem.ncbi.nlm.nih.gov/compound/Nodularin#section=Chemical-and-Physical-Properties (accessed on June 30, 2020).

Picardo, M., Filatova, D., Nuñez, O., Farré, M., 2019. Recent advances in the detection of natural toxins in freshwater environments. Trac. Trends Anal. Chem. 112, 75–86. https://doi.org/10.1016/j.trac.2018.12.017.

Raeke, J., Lechtenfeld, O.J., Wagner, M., Herzsprung, P., Reemtsma, T., 2016. Selectivity of solid phase extraction of freshwater dissolved organic matter and its effect on ultrahigh resolution mass spectra. Environ. Sci. Process Impacts 18, 918–927. https://doi.org/10.1039/C6EM00200E.

Real, M., Gámiz, B., López-Cabeza, R., Celis, R., 2019. Sorption, persistence, and leaching of the allelochemical umbelliferone in soils treated with nano-engineered sorbents. Sci. Rep. 9, 9764,. https://doi.org/10.1038/s41598-019-46031-z.

Roy-Lachapelle, A., Vo Duy, S., Munoz, G., Dinh, Q.T., Bahl, E., Simon, D.F., Sauvé, S., 2019. Analysis of multiclass cyanotoxins (microcystins, anabaenopeptins, cylindrospermopsin and anatoxins) in lake waters using on-line SPE liquid chromatography high-resolution Orbitrap mass spectrometry. Anal. Methods 11, 5289–5300. https://doi.org/10.1039/C9AY01132C.

Schenzel, J., Schwarzenbach, R.P., Bucheli, T.D., 2010. Multi-residue screening method to quantify mycotoxins in aqueous environmental samples. J. Agric. Food Chem. 58, 11207–11217. https://doi.org/10.1021/jf102737q.

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ. Sci. Technol. 48, 2097–2098. https://doi.org/10.1021/es5002105.

Schymanski, E.L., Singer, H.P., Slobodnik, J., Ipolyi, I.M., Oswald, P., Krauss, M., Schulze, T., Haglund, P., Letzel, T., Grosse, S., et al., 2015. Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis. Anal. Bioanal. Chem. 407, 6237–6255. https://doi.org/10.1007/s00216-015-8681-7.

Servei Meteorològic de Catalunya https://www.meteo.cat/wpweb/climatologia/el-clima-ahir/historic-cartografia-climatica/historic-de-mapes-de-temperatura-mensual/. Availabe online: (accessed on).

Takagi, H., Ishiga, Y., Watanabe, S., Konishi, T., Egusa, M., Akiyoshi, N., Matsuura, T., Mori, I.C., Hirayama, T., Kaminaka, H., et al., 2016. Allantoin, a stress-related purine metabolite, can activate jasmonate signaling in a MYC2-regulated and abscisic acid-dependent manner. J. Exp. Bot. 67, 2519–2532. https://doi.org/10.1093/jxb/erw071.

Testai, E., Buratti, F.M., Funari, E., Manganelli, M., Vichi, S., Arnich, N., Biré, R., Fessard, V., Sialehaamoa, A., 2016. Review and analysis of occurrence, exposure and toxicity of cyanobacteria toxins in food. EFSA support. publ. 13, 998E,. https://doi.org/10.2903/sp.efsa.2016.EN-998.

Turner, A.D., Dhanji-Rapkova, M., O'Neill, A., Coates, L., Lewis, A., Lewis, K., 2018. Analysis of microcystins in cyanobacterial blooms from freshwater bodies in England. Toxins 10, 39,. https://doi.org/10.3390/toxins10010039.

Yen, H.K., Lin, T.F., Liao, P.C., 2011. Simultaneous detection of nine cyanotoxins in drinking water using dual solid-phase extraction and liquid chromatography-mass spectrometry. Toxicon 58, 209–218. https://doi.org/10.1016/j.toxicon.2011.06.003.